THE FUNCTION OF THE NUCLEUS OF THE LIVING CELL

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Contents

I. Introduction.

It is a well recognized fact that the science of physiology, and in fact all biological science, has not attained to that degree of exactness which is characteristic of the sciences of physics and chemistry. To the question, how may physiology be made a more exact science, there are as many answers as there are methods of investigation; but there is one line of attack, based upon simple logic and upon the analogous development of those sciences which are nearer the goal, which may rightly claim special attention.

He who surveys the development of the sciences of physics and chemistry, and especially the rapid advances of recent years, must be struck with one phase of this development. With the formation of the atomic theory, both sciences were able to make a great advance. They were now in possession of a unit by means of which they could conceive of the occurrence of phenomena, explain them, and make conjectures and even predictions. And with the subsequent intensive study of this unit of matter, the advances in physics and chemistry were marvelously accelerated, and their progress was roughly parallel to the progress in the study of the atom. Deprived of the conception and knowlege of the atom, modern chemistry and modern physics could

scarcely be called sciences. In a quite analogous manner, he who surveys the development of biological science can not but be struck by the great advances which followed the formation of the cell theory. What the atom is for physics and chemistry the living cell is for biology: and it is not surprising that the discovery and identification of the unit of living matter was followed by just as marvelous a development in the science of living matter as succeeded the conception of the unit of non-living matter. But when we turn to look for the intensive study of living matter, and the scientific advancement which would just as surely follow this study, we are doomed to some disappointment. The intensive study of the living cell has for years been limited to one method: namely, the study of the dead cell with the hope of finding out more about the living cell. The results which have been obtained by the use of this method have been very instructive as far as they have gone, but it is to be expected that this method alone would have its limitations; and in fact it would seem that this aspect of cell study has already been pushed as far as may profitably be done. It is a logical conclusion that the biologist must now turn his attention to the more difficult task of the direct study of the living cell: but may he not feel confident that these studies will be rewarded by a progress quite equal to the recent progress in physical science?

All of the phenomena occurring in living matter, the phenomena which it is the avowed purpose of physiology to study, are ultimately referable to changes occurring in the living cells of which it is composed. Many of the phenomena occurring in living matter, such as secretion, movement, irritability, and growth, can readily be referred to the more fundamental changes in the living cells; but the attempt to interpret any single property of a living cell, such as growth, division, contraction, in terms of physics and chemistry, or in any other way, has thus far led to no result satisfactory to the minds of scientific inquirers. Nevertheless, it seems that this is the task which lies before the science of physiology on its way toward becoming an exact science: the task of interpreting and explaining the fundamental properties of living cells.

One who turns his attention to the direct study of the living cell may be at a loss how to begin. It is undoubtedly owing to the technical difficulties of the subject that it has not already been further advanced. We are dealing with tiny objects of an almost inconceivably delicate constitution, morphologically very complex, and chemically of far greater complexity than any other known substance: and to these mysterious little objects are to be attributed all of the various phenomena of living matter: for all of the activities of living matter may be ultimately referred

to activities of its constituent units, the living cells. Nothing will admit of so little manipulation, and all of the lore of physical science must be drawn upon for the careful disentanglement of the maze of phenomena exhibited by a single living cell. A tiny speck of jelly, more than three-fourths water, it may move, feed, digest, grow, reproduce, and respond to all manner of changes in its surroundings, but the slightest change in a single condition of its environment may snuff out its life.

But since we must begin somewhere, and since it is the phenomena of the living cell which we are to study, let us start by dividing these phenomena roughly into two classes:
1. Those phenomena which are characteristic of living cells in general, and which are exhibited by most or all living cells. Such are respiration, growth, irritability.
2. Those phenomena which are peculiar to certain types of living cells, and not exhibited by the great majority of cells. Such are ameboid and ciliary movement, photosynthesis, secretion, and perhaps contractility.

Now while both of these classes of phenomena must be studied and are of great importance on account of their widespread occurrence and significance, it seems that in the present state of our knowlege of living cells, ignorant

as we are of those general phenomena which all living cells exhibit, we could most profitably turn to the study of those phenomena common to most or all living cells. The experiments presented in this paper represent such a study.

In various branches of biological science, progress has been made by first studying structures and then attempting to discover the significance of these structures, and while there is no intrinsic reason why it should, it seems plausible that cell physiology may advance along the same lines. At any rate, it can not be denied that the problem of the function of the ~~cell~~ nucleus, the most characteristic structure of the cell, is one of the most alluring problems of cell physiology, and as such it has excited much speculation, and been the subject of some experimental work.

Before reviewing the experiments of previous workers in this field, it might be well to call attention to a few matters of common knowlege and observation which bear upon the problem. The most obvious fact with regard to the nucleus is that it almost invariably occupies a central position in the cell, and no biologically trained man, accustomed as he is to regard the smallest acts of nature as significant, would fail to appreciate the importance of this fact. Reflection upon the central position of the nucleus leads to the conclusion that the nucleus must

play a central role in the activities of the cell, and that it must be of great importance in the life and activities of the cell as a whole. Moreover, when the nucleus does not occupy a central position, the ex-centric position which it does occupy may also be of significance. In basement membranes and other epithelial cells of higher organisms the nucleus is found toward the side of the cell which is closest to the blood supply, and this, together with the fact that in other cells the nucleus may be seen in close proximity to, or even engulfing nutritive material, leads to the conclusion that the nucleus must play an important role in the nutrition of the cell. The careful provision which is made for the equal division of the nucleus, and apparently for even parts of the nucleus, when the cell divides: the complex but exact changes which the nucleus undergoes in preparation for reproduction; the careful allotment of certain nuclear material from each parent for the formation of the new individual, and the subsequent union of this nuclear material from each side, all go to show that the nucleus is important in the properties and characteristics, the individuality, and even the existence of the cell. Nature has also furnished us, in the case of the red blood corpuscle, with the interesting experiment of a cell without a nucleus, and we know that its life is

limited, its days are numbered, just as are those of the medullated nerve fiber which has been severed from its nucleus.

Such were the facts which the older biologists had at their disposal in the attempt to solve the problem of the function of the cell nucleus; and these facts together with certain subsequent experimental developments have led to the formation of two main theories with regard to the function of the cell nucleus. The first and oldest theory I have been unable to find clearly stated by any earlier writer than Claude Bernard: it asserts that the nucleus is responsible for the building up processes, the synthetic or anabolic activities of the cell. "Il semble que la cellule qui a perdu son noyau soit stérilisée au point de vue de la génération, c'est-à-dire de la synthèse morphologique, et qu'elle le soit aussi au point de vue de la synthèse chimique, car elle cesse de produire des principes immédiats, et ne peut guère qu'oxyder et détruire ceux qui s'y étaient accumulés par une élaboration antérieure du noyau. Il semble donc que le noyau soit le germe de nutrition de la cellule; il attire autour de lui et élabore les materiaux nutritifs." ....."Le protoplasma circumnucléaire, d'autre part, renfermerait tous les produits de l'élaboration synthétique du noyau, c'est-à-dire les principes immédiats destinés à se detruire et

s'oxyder." (Bernard, 1897, p523). The second and more recent theory is attributed to Jacques Loeb, a well known advocate of the theory, It asserts that the nucleus is the center of oxidations of the cell, the organ where the oxidative processes are most prominent and rapid. " It seems to me therefore, that all the facts which are known thus far very naturally support the idea that the nucleus is the organ of oxidation of living matter: and that fragments of cells without a nucleus are not able to regenerate because their oxidative activity has fallen to too low a point. Such pieces die slowly from asphyxia." (Loeb [illegible]).

While it may be that both of these theories fall wide of the mark, it is well, in reviewing the experimental data at hand, to bear in mind their relation to the two views: that the nucleus is the synthetic organ or the oxidative organ of the cell.

In seeking an experimental proof of the function of the cell nucleus, only one method has been extensively used: that of dividing the living cell into two parts, and making a comparative study of the part with a nucleus and the part without a nucleus, the differences being attributed to the presence or absence of the nucleus. The other exper-

iment which suggests itself, that of isolating the nucleus from a cell and studying the isolated organ, has been unsuccessful, owing, no doubt, to the very rapid death of the nucleus which occurs after its removal from the cell.* But the fruitful and suggestive results of studying the chemistry of the isolated organ are well known.

Altho the nucleus has, since its discovery by Robert Brown in 1831, been the subject of much morphological investigation, most observations and experiments of a physiological nature upon this subject are of a comparatively recent date. The earliest experiments appear to be those of K. Brandt (1887) upon the rhizopod Actinosphaerium Eichhornii. After division of this organism, he observed that the pieces which contained nuclei regenerated to complete individuals, while those which lacked a nucleus always died without regeneration. Similar observations were made upon plant cells by Schmitz (1879). Upon rupture of the membranes of cells in the alga Valonia utricularis and Siphonocladus Wrisbergi, the protoplasm rounded up into little globules, some of which contained nuclei and some did not. While

*The author has frequently observed a nucleus, which had been isolated from an ameba by destruction of the cytoplasm, undergo a rapid darkening, becoming distinctly more opaque in a few seconds. While the significance of this phenomenon is not understood, it may be the result of an oxidative change terminating in the death of the organ.

the nucleated pieces soon formed new membranes and continued to live, the non-nucleated pieces died without regenerating a membrane. These observations were extended and confirmed by the careful experiments of Nussbaum (1885). With a fine needle he divided the infusoria Oxytricha and Gastrostyla into a nucleated and non-nucleated piece. The non-nucleated piece died in the course of a few days, without any manifestation of regeneration; but the nuclear part was regenerated into a complete cell, and continued to grow and reproduce by division. From these experiments it is evident that for the phenomena of growth and division, and for the regeneration of lost parts, the nucleus is absolutely essential: and upon these facts all subsequent investigators are agreed. The observations of Gruber (1884) upon Spirogyra: those of Balbiani (1892) and Verworn (1888) upon many infusoria: those of Hofer (1890), Stolc (1910), and the author upon Ameba proteus: and many other direct and indirect observations lead inevitably to the conclusion that without the nucleus cell growth and cell division as well as the regeneration of lost parts are absolutely impossible. Whatever additional function the nucleus may have, its relation to the phenomena of growth is beyond question.

Altho there is no such uniformity of opinion with regard to the part played by the nucleus in other activ-

ities of the cell, the disagreement is, in some cases, readily understood. The phenomena of movement, being the ones most readily observed, have been studied most extensively. Nussbaum observed that in the enucleated pieces of infusoria the cilia continued to beat up to the onset of death. In like manner Balbiani and Verworn observed that the normal activity of the cilia continued after the nucleus had been removed, until death changes set in. With regard to ameboid movement, however, there is some difference of opinion. Gruber observed that enucleated pieces of Ameba proteus ceased making normal movements soon after the operation, and this was confirmed by Hofer (1890) and by Willis (1916). Stolc (1910) however, apparently using a different variety of Ameba proteus, observed normal movements for days after removal of the nucleus. But the most striking experiments upon the influence of the nucleus upon normal movement were made by Verworn, using Lacrymaria olor, in which the movements are very complex. This flask-shaped ciliate extends its long neck and waves it about, the cilia upon the head end beating actively, and then suddenly retracts like an elastic band, to repeat the performance later. If stimulated it swims away. Non-nucleated parts of this organism, regardless of which part is selected, exhibit the same characteristic and

complex movements and response to stimuli as when they formed a part of the intact organism. We are thus led to conclude that altho removal of the nucleus does in some cases interfere with normal movement, at any rate perfectly normal movement and irritability are quite possible without the presence of the nucleus.

Closely related to the phenomena of movement are the phenomena of respiration, and there are many interesting observations which bear more or less directly upon the relation of the nucleus to cell respiration. Aside from the fact that, in aerobic organisms, normal movement implies normal respiration, the activity of the contractile vacuole indicates that respiration is not interfered with by removal of the nucleus. There is a general agreement that the function of the contractile vacuole is that of a respiratory organ, aiding in the removal of carbon dioxide from the interior of such large cells as protozoa. When a protozoon is divided into two parts. the part which lacks a contractile vacuole soon forms one, regardless of whether it contains a nucleus or not, (Balbiani, Hofer, Stolc, Penard). The vacuole contained in the non-nuclear fragment pulsates with about the same frequency as that in the nuclear fragment, but gradually becomes slower with

the inevitable changes of death (Balbiani, Hofer, Stolc).*

That the non-nuclear red blood cell respires is a well known fact, and Tashiro (1917) has recently demonstrated that the non-nuclear nerve fiber produces carbon dioxide and that this carbon dioxide production is increased by stimulation and decreased by anesthetics. Moreover, measurements of the respiration of nerve ganglia showed that the part of the cell which contained the nucleus produced no more carbon-dioxide than the non-nuclear portions, and possibly not as much.

Loeb (1905), however, basing his opinion in part upon the supposed presence in the nucleus of nucleo-proteins which contain iron, suggests "that the nucleus is the organ of oxidation of living matter; and that fragments of cells without a nucleus are not able to regenerate because their oxidative activity has fallen to too low a point." But he adds "I do not beleive that without the nucleus all processes

*The regeneration of the contractile vacuole does not, as might at first sight appear, belie the fact stated above: that regeneration of parts is impossible without the nucleus; for the contractile vacuole is not to be looked upon as a structural element of the cell, such as the oral groove. but rather is its appearance to be regarded as the expression of a certain condition in the protoplasm. The presence of certain granules in the locality where the vacuole has repeatedly appeared (Metcalf, 1910) may be the result rather than the cause of its appearance at this point, for the granules evidently accumulate at the edge of the formed vacuole.

of oxidation cease in the protoplasm." In support of this view, Osterhout (1917) has recently published some interesting observations upon the cells of the leaf of the Indian Pipe. He finds that these cells become dark when they are injured, owing to the oxidation of a pigment which they contain; but that the darkening, and hence the oxidation, occurs most readily in the nucleus. From this he concludes that oxidation is most rapid in the nucleus of the uninjured cell. These experiments will be discussed later.

That it is the protoplasm rather than the nucleus which is concerned in respiration is indicated by the experiments of Demoor (1895). Under conditions which are known to depress oxidations, such as cold, and lack of oxygen, or the presence of anesthetics, Demoor brot the protoplasm of spirogyra cells into a condition of inactivity; but the activity of the nucleus continued as shown by its repeated divisions. In like manner the nucleus of a frog's leucocyte continued its ameboid movements after the cytoplasm had been rendered inactive by chloroform.*

* Child (1915) calls attention to the fact that anesthetics affect most quickly those regions which have the most rapid respiration.

With regard to the ability of the non-nucleated cell to secrete, there are some interesting observations upon Ameba proteus. In normal movement as seen upon a glass slide, this organism is attached to the bottom and can thus move from place to place. This ability to stick to the bottom, as well as to stick to food particles, seems to depend upon a secretion which is present upon the surface of Ameba. Without this secretion the ameba is not able to move from place to place, but can only stretch out pseudopods in different directions. That the production of this secretion is in some way dependent upon the presence of the nucleus is shown by the fact that the secretion is absent shortly after the removal of the nucleus, as shown by Hofer.

Further evidence upon the question of the ability of the enucleated cell to produce secretions is found in experiments upon digestion. Hofer, Stolc, and others have observed more or less normal digestion of food particles ~~soon~~ after removal of the nucleus; but observations male upon the digestion of particles ingested before enucleation, and to which the digestive secretions have already been added, are little more to the point than observations made upon digestion in a test tube. Some crucial experiments were performed by Hofer, however. Hofer removed the

nucleus from ameba proteus as soon after the ingestion of a paramecium as possible. If the operation was performed immediately the paramecium was not killed, but escaped, owing doubtless to the lack of certain secretions. But even when the operation was not performed until the death of the paramecium, the digestion was much more slow and incomplete than normal. Hofer concludes that "protoplasm can produce secretions only with the aid of the nucleus."

Other substances than digestive secretions are no longer formed after the removal of the nucleus. Thus Klebs (1887) observed that when cells of Spirogyra were treated with strong sugar solutions, and small globules of protoplasm were formed as the result of the plasmolysis, only those fragments which contained nuclei made new cellulose membranes. Later Verworn (1888) showed that pieces of Polystomella crispa, a protozoon which secretes about it a small shell of lime, were able to form a new shell provided they contained a nucleus, but the pieces which lacked a nucleus, tho showing the normal ameboid movement, were quite unable to form a new shell. The relation of the nucleus to the formation of these substances is further born out by the observations of Haberlandt (1887): that whenever there is a localised formation of new cell wall

in plant cells the nucleus is found in close juxtaposition to the point of this formation. Korschelt (1889) also observed that in the egg cells of the water beetle, Dytiscus marginalis, where the eggs are supplied with nutritive granules by surrounding cells, the nucleus sends pseudopod like processes in among these granules. There are also numerous observations of an exchange of granules between nucleus and cytoplasm.

In apparent contrast to these observations, Palla (1890) found that in the root hairs and pollen tubes of some phanerogamic plants, enucleated bits of protoplasm, when selected from a growing part, were able to form a new cell wall: the "cellulose reaction." These observations may not, however, be as contradictory as they seem, for the importance of selecting a growing part suggests that we may be dealing with an after effect of the nucleus: that the formative substances produced by the nucleus may have been already present in the protoplasm at the time of its separation.

Such an explanation is not applicable, however, when we come to the formation of starch. In the experiments of Klebs quoted above, the interesting discovery was made that non-nuclear protoplasm, provided it contained chlorophyl, was quite capable of forming starch in the light.

These observation were later confirmed by Gerassimoff (1890). This is the one outstanding case of an organic synthesis which occurs quite independently of the presence of a nucleus. But it is to be remembered that this synthesis is performed by certain specific organs, the chloroplasts, which are themselves not unlike nuclei; and in fact that one step in this synthesis may be performed by chlorophyl which has been extracted from the leaves (Usher and Priestly).

While the series of experiments which have been reported show clearly that the nucleus is necessary for the proper performance of certain functions by the protoplasm, and for the growth and continued life of the protoplasm, the converse fact must not be overlooked: that the protoplasm is necessary for the performance of the functions of the nucleus, and even for its continued existence. Thus in the formation of new substances by the cell, the activity of the cytoplasm is quite as essential as that of the nucleus. This is strikingly shown by the experiments of Demoor cited above. When by means of cold, narcotics, and the like, he inhibited the activity of the cytoplasm, the activity of the nucleus continued, but the formation of a new cell wall was prevented. Moreover, Verworn (1902) showed that nuclei isolated from Thalassicolla nucleata soon disintegrated to

a granular mass. The nuclei also died if they were injected into normal or enucleated protoplasm, which indicated that the nucleus is very quickly injured when removed from its protoplasm.

II. Method.

Of the different methods which have been employed to solve the problem of the function of the cell nucleus, the one which has given the most illuminating results is the method of extirpation: removing the nucleus and comparing the enucleated cell with a normal nucleated cell. In order that the nucleus may be removed from a cell, or that a cell may be cut in half, it is necessary or expedient that a cell of considerable size be selected; but at the same time it must be a cell which can be kept alive or cultivated for several generations if necessary. The cells which meet these requirements most exactly are certain unicellular organisms, certain protozoa. Among such a large and diverse group of organisms, it is not difficult to find one which is peculiarly suited for the problem at hand. For the experiments which follow, Ameba proteus was selected. Owing to the slow movements and absence of shell in this organism, it can readily be cut in half: or if desired the nucleus may, after some practice, be removed with as little as one tenth or less of the protoplasm. Moreover, the lack of differentiation in Ameba is a decided advantage, for we are certain that in removing part of the protoplasm

with the nucleus, we are removing no important organ of the cell. The one disadvantage in the use of this fascinating living cell is the difficulty of cultivating it. Like all rhizopods amebas are difficult to raise, but many investigators have succeeded in keeping healthy cultures in the laboratory for years.

In view of the difficulties which are so often met in cultivating amebas, it may not be out of place to add one more method to those which have been described for rearing these valuable experimental animals. It is almost literally true that each investigator has a method of raising amebas, but that no one can use it except himself. This is not surprising when it is seen that "water" is employed in making the cultures, with little regard for its purity. While amebas may flourish in the "water" of one locality, they may quickly die in the water of another locality. This difficulty was avoided by the use of distilled water.* The method which follows is based upon one which has been employed by Miss Hyman of the University of Chicago.

* The prejudice of many biologists against the use of distilled water, and the prevalent notion that it is toxic. are not supported by experiments upon the toxicity of distilled water. (Daniel . 190[illegible]).

A small amount of hay, including both stalks and leaves, is cut into pieces about three inches long; four grams of this material is then placed in a large beaker. To this is added 0.2 gram of dry bread crumbs and 500 cc of distilled water. The material is boiled for a few minutes, and then poured into flat dishes to a depth of 1 to 2 cms. The dishes are covered to keep out dust, and if water evaporates from them it can be replaced by distilled water. After the infusion has cooled, several pipettes full of fluid are added from a culture which contains healthy amebas, or from a pond or stream where amebas are found. In doing this, care is taken to avoid taking up any of the sediment, for it is the sediment rather than the super-natant fluid which is apt to contain enemies to Ameba. This innoculation may be repeated, and in the course of two weeks the culture becomes cloudy with bacteria, zoogloea, and small ciliates and flagellates: the food of Ameba. A small dish of the culture fluid is now seeded with amebas, being careful to avoid large organisms such as crustacea and worms. If living amebas are not present a week later, more of the fluid containing amebas should be added. This culture is used to innoculate the rest of the infusion. The addition of a little hay occasionally will keep the amebas present in great numbers. The room should not be allowed to become very hot or very cold.

The amebas were transferred to different media by means of a fine glass capillary pipette, the sharp end of which had been made smooth by passing thru a flame. In most of the experiments the animals were kept in drops of fluid upon slides with a depression at each end. The slides were kept in a moist chamber in a room whose temperature did not vary greatly from 20°C. For cutting the amebas in half, or cutting the nucleus away from the rest of the cell, a fine glass needle was drawn off at a slight angle from the end of a glass rod. The cells were divided under a binocular microscope.

Since the animals would not live long in the tap water of this city, a search was made at the outset for a solution in which they could be kept in healthy, active condition. Ten amebas were removed from a culture, freed from food material and debris, and transferred to the solution to be tested. Each day they were examined and transferred to a fresh solution, the experiment being continued until all of the animals were dead. It appeared at first that distilled water was the most satisfactory medium that could be obtained. Thus ten amebas lived (without food) for an average of 13.2 days in distilled water, whereas animals kept in tap water, for example, soon assumed an abnormal appearance, and could seldom be kept alive longer than one week. But it was later discovered that

the distilled water was greatly improved by the addition of one tenth the volume of spring water. Not only was the average life somewhat longer, ($14\frac{1}{2}$ days), but the condition of the animals during this time was greatly improved. In the distilled water, the animals soon cease to be attached to the bottom, and are hence unable to move from place to place in the normal manner; but in the "10% spring water", after it became saturated with air, they remained attached up to a few days before death. The spring water used contained a trace of calcium, but not as much as was contained in the tap water. In the following experiments the amebas were, unless otherwise noted, kept in 10% spring water.

Relation of the Nucleus to Movement.

The movement of normal amebas in a suitable medium, such as the dilute spring water, when placed upon a glass slide, was found to be of the limax type. The ameba is attached to the slide by means of some sticky secretion, and flows steadily forward, sending out pseudopods at the anterior end, often from alternate sides. Frequently amebas are found which are not attached to the slide, and such amebas may project pseudopods in any direction, but are unable to move from place to place. These animals will in time, however, adhere to the slide, unless they are abnormal or the medium is unsuitable. When these active amebas are stimulated roughly, as by pressure with a glass needle, they retract into a more or less spherical shape, and small droplets of protoplasm may be seen projecting from the surface.

The effect of amputation of the nucleus upon the movement of the cell may be seen from the following typical experiment:

3:21 P.M.- An active ameba which was attached to the slide was divided into two approximately equal parts, the nucleus remaining in the posterior half. Both parts went into the typical condition of stimulation.

3:25 P.M.- Both pieces have put out pseudopods and are active, but the non-nuclear part, which was cut from the anterior end, is much more active than the nuclear and posterior part.

3:27 P.M.- Both pieces are moving in the typical limax

fashion, but the movement of the non-nucleated piece has become perceptibly slower.

5:28 P.M.- The non-nucleated piece ceases its progressive movements and slowly retracts into a somewhat corrugated sphere. Only an occasional very blunt pseudopod is now produced, and slight agitation shows that the fragment is no longer attached to the slide. The nucleated piece differs from a normal ameba only in size.

The retraction into the spherical shape is an invariable phenomenon, and is of such a definite character that the time of its appearance may be accurately determined. Thus in 15 amebas which were so divided that the nucleus remained in the posterior half, the retraction occurred at an average of 9 minutes after the operation. To the observer it resembles strongly the response to stimulation, except for the absence of the protoplasmic droplets mentioned above. The protoplasm is still capable of movement, as is shown by transferring it to another medium, such as distilled water, to which it responds by a change of shape.

This effect of amputation of the nucleus upon movement is not always permanent. If a number of amebas are cut in half, and the nucleated and non-nucleated pieces observed each day, on the first or second day after the operation it will be noticed that many of the non-nucleated fragments are moving in the typical limax fashion, and in fact there is sometimes no great difference between the

movements of the nucleated and non-nucleated pieces. Slight agitation, however, shows that the non-nuclear amebas are scarcely attached to the slide; the slightest disturbance serves to dislodge them. This fact, which must be due to the failure to produce the sticky secretion, is probably partly responsible for the difference in movement between the nuclear and non-nuclear cells.

The fact that a non-nucleated ameba may, under any conditions exhibit normal movements, justifies us in concluding that the nucleus is not necessary for movement. In ameba, however, movement is affected in some indirect way by removal of the nucleus.

The Survival of the Non-Nucleated Cell.

All observations made upon cells which have been deprived of their nuclei prove conclusively that non-nucleated protoplasm is destined to grow without growing or dividing. But in most cases, particularly in ameba, it has been impossible to get this protoplasm to take in food, and even that food which is already included in the protoplasm is but imperfectly digested. Consequently it is fairer to compare the survival of an enucleated cell with one which has been isolated without food, rather than with an actively feeding cell. When this is done, it is found that the non-nucleated cell lives almost as long as the nucleated one, provided there is no difference in size. If an ameba is cut into unequal parts, the nucleus, owing to its central position, will usually be found in the larger part. Since large fragments live longer than small ones, special care must be taken to avoid a difference in size.

Fifteen amebas were cut in half, and both halves were kept without food in either distilled water or in water taken from the culture. The number of days which each piece lived after the operation is given in the following table:

Medium	Survival of nucleated part	Survival of non-nucleated part
Distilled water	7 days	6 days
"	7	7
"	10	6
"	9	4
"	7	4
"	5	3
"	9	6
Culture water	7	5
"	8	6
"	6	6
"	9	7
"	11	8
"	7	8
"	7	7
"	7	7
Average	7.7 days	6days

It will be noted that in some cases the non-nucleated fragments lived as long as those which contained nuclei. In fact, by cutting the ameba in such a way that the piece containing the nucleus was smaller than the piece which lacked it, the non-nucleated cell could be made to outlive the nucleated one. Thus ten amebas were divided in such a way that the nucleated piece was from one-half to one-fourth the size of the other. The results are tabulated below :

Survival of nucleated part	Survival of non-nucleated part
6	10
7	7
3	13
6	5
5	6
6	7
7	7
6	8
7	7
6	10
Average- 5.7 days	8 days

We may conclude that a cell deprived of its nucleus may survive as long as a cell deprived of food.

The Cultivation of Ameba by Substances in Solution.

The apparent resemblance of enucleated amebas to amebas deprived of food suggested that some of the phenomena exhibited by the non-nucleated cell might be the result of starvation, and have no direct connection with the absence of the nucleus. Accordingly, an attempt was made to supply the amebas with adequate food substances. Since it was not possible to get the enucleated organisms to take in food particles, an effort was made to provide a medium in which amebas could be nourished by the absorption of substances in solution.

It has been pointed out that no artificial synthetic medium has ever been provided which was adequate to nourish an animal cell (Burrows and Neymann, 1917), although the blood and lymph apparently constitute a natural synthetic medium for the cells of higher organisms. Even cells cultivated in vitro depend upon the autolysis of neighboring cells for their nourishment, and the attempt to cultivate protozoa in nutritive organic media has met with complete failure (Biedermann, 1916, p278: Doflein, p 398). In the light of recent experiments in nutrition, however, it seems possible that such negative results may be due

to the failure to supply certain substances which are necessary for proper nutrition. An attempt was therefore made to provide an adequate medium for the primitive animal cell, Ameba proteus.

Since amebas were found to move more normally and to live longer in distilled water to which had been added one-tenth the volume of spring water, this "10% spring water" was used in making up all solutions. Various carbon compounds, including several sugars, were now tested with the hope of finding a source of energy.

The method used was as follows: A number of amebas were selected from the same culture, and freed from food and bacteria by transferring repeatedly to fresh solutions of 10% spring water. They were then transferred to slides, placing 10 in 10% spring water as a control, and 10 in each of the solutions to be tested. The animals were examined and counted each day, washed in water, and transferred to fresh solutions to prevent the development of bacteria. The most promising substances found were the hexoses, glucose and levulose, the effect of which may be seen by the following experiments:

Date	Number of amebas living		
	Water	1% Glucose	1% Levulose
Feb. 7	20	20	20
8	20	20	20
9	18	20	20
10	15	20	20
11	15	20	20
12	15	20	20
13	13	20	20
14	13	20	20
15	13	20	20
16	13	20	19
17	13	20	19
18	13	20	18
19	13	20	18
20	11	18	17
21	11	17	17
22	11	17	15
23	9	17	15
24	7	17	15
25	6	16	14
26	6	16	12
27	4	12	11
28	3	12	8
Mar. 1	2	11	8
2	2	10	2
3	2	6	0
4	1	4	
5	1	3	
6	1	0	
7	1		
8	0		
Average life	12.6 days	21.8 days	19.4 days

Not only did the amebas live longer in the sugar solutions, but during the early part of the experiment they were much more active. At the end of a week, however,

many of the amebas began to take on an opaque appearance, and ceased normal movements. The substance which was formerly a food now acts as a poison. Child (1915) has shown that susceptibility to poisons increases during starvation.

Altho these cultures were not sterile, it was possible to keep the bacteria from becoming numerous, and thus prevent their becoming an appreciable source of food for the amebas.

Having found a carbohydrate food for Ameba, a search was now made for a source of nitrogen. In such a primitive cell, the possibility that simple compounds of nitrogen may be used, at once suggests itself. Of various compounds of nitrogen which were tried, the best results were obtained with ammonium nitrate and urea; and of these two, urea was much the better, owing to its comparatively low toxicity. The effect of these substances is seen in the following experiment. (The comparatively short life of the animals is to be attributed to the use of an inferior culture).

Date	Number of amebas living: 1% Glucose	1% Glucose +0.1% urea	1% Glucose + 0.01% NH_4NO_3
Feb. 24	10 days	10 days	10 days
25	9	10	8
26	9	11	8
27	9	12	8
28	6	12	8
Mar. 1	5	12	8
2	5	12	8
3	4	12	8
4	4	12	8
5	4	12	8
6	4	10	8
7	4	10	8
8	4	10	8
9	4	8	7
10	4	6	7
11	4	6	7
12	4	6	4
13	3	5	3
14	2	4	2
15	1	3	2
16	1	3	1
17	1	3	0
21	0	1	
22		1	
27		0	
Average life	9.1 days	16.6 days	12.9 days

That these compounds are of some use to the amebas is indicated by the increased length of life when they are added to the solution; but that they are not an adequate source of nitrogen is indicated by the fact that the animals eventually die and that no growth can be

detected. The shrinkage caused by the glucose would render the detection of growth difficult even if present.

Of special interest, however, are the three cases of cell division seen in the urea solution. Altho hundreds of amebas have been kept under daily observation until they were completely disintegrated, no single case of cell division was ever observed unless food substances were supplied: and with two possible exceptions, no division was observed unless a source of nitrogen was added. On the other hand, divisions have been repeatedly observed in solutions containing urea or certain amino acids. That the division is not simply the result of the stimulating action of the urea upon the protoplasm is shown by the effect of urea solutions to which no glucose is added. In such solutions, the amebas not only do not divide, but they die more quickly than in water alone. Urea in the absence of glucose is simply a mild poison, but when urea and glucose are used together, the animals live longer, have a more normal appearance, and may even reproduce. The necessity of using the two substances together indicates that they are built up or combined to form some more complex substance which is of use to the organism.

If this is a true case of organic synthesis, it would

be interesting to see whether it occurs in the cell which has been deprived of its nucleus, in order to throw light upon the supposed synthetic function of the nucleus. This point is taken up later.

Having failed to obtain growth with simple compounds of nitrogen, the organisms were now supplied with amino-acids. The use of single amino-acids was not promising. Some reproduction was obtained with a saturated solution of tyrosine, but the animals soon died. A mixture of amino-acids was then prepared in the following way:

Five grams of Hammarsten's casein was heated for twenty hours with 100 cc of a molecular solution of sulphuric acid. The hydrolysis was performed on a water bath in a flask fitted with a reflux condenser. At the hydrolysis, when the solution no longer gave the biuret reaction, a saturated solution of barium hydroxide was added until the reaction was slightly alkaline, and the solution boiled to remove ammonia. Dilute sulphuric acid was added until very slightly acid, and the solution filtered hot. The barium sulphate precipitate, which had adsorbed much of the humus substance, was not washed. The solution was exactly neutralized with sodium hydroxide, and the resulting solution, whose volume was 700 cc, had the following properties:

A clear, straw-colored fluid, with a bitter-sweet taste. The reactions for barium, calcium, iron, reduced sulphur, and tryptophane were negative; phosphate was present, and the xantho-proteic reaction was positive. When exposed to the air, bacteria develop with remarkable rapidity. The solution was not toxic to amebas, even when evaporated to one half the volume, but evaporation to one fifth the volume resulted in a solution which was slightly toxic.*

This solution of amino acids is lacking in glycine, which is absent in casein, and in tryptophane and cystine, which have been destroyed.

In cultivating amebas in this solution, it was difficult to prevent the development of bacteria, but a method was devised which served to exclude them almost completely. The amebas were washed five to ten times in sterile water, and the slides upon which they were kept were boiled. The culture solutions were kept in small flasks fitted with capillary tubes. By inverting the flask and warming with the hand, a few drops could be placed upon the slide. After using, the flask was boiled

* The toxicity of amino acids observed by Burrows and Neymann (1917) upon embryonic chicken cells was probably due to the relatively concentrated solutions used.

and a test tube placed over its neck. The cultures were examined daily, the amebas washed in sterile water, and transferred to new solutions.

The hydrolysed casein solution proved to be a fairly good medium in which cases of cell division were occasionally observed, but there was never any growth, and the changes which preceded death could be seen in about ten days. The addition of glucose resulted in some improvement, but it did not prevent death.

Number of amebas living.

Date	Hydrolysed casein	Hydrolysed casein + 0.2% glucose	
May 1	5	5	
2	5	6	
3	6	6	
4	6	6	
5	6	7	
6	6	8	
7	6	8	
8	Dying	8	
9	Discontinued	8	
10		8	
11		8	
12		8	Amebas healthy
13		8	Somewhat abnormal
14		7	
		Dying	Exp. discontinued

Since this death might be due to the absence of some foodstuff, various substances were added to the "diet".

The addition of various salts was tried, but no improvement was observed. Cystine and tryptophane, so important in the nutrition of higher animals, were added to the solution in concentrations which were shown not to be toxic. A small amount of milk was added to provide the unknown "accessory" food substances and certain salts.

Date	Hydrolysed casein + 1% glucose + 0.1% tryptophane + 0.03% cystine + 0.2% milk
May 23	5
24	5
25	6
26	6
27	6
28	6
29	6
30	6
31	6
June 1	6
2	6
3	5
4	3
5	3
6	1
7	0

No adequate synthetic medium was found for Ameba proteus. The failure may be due to some peculiarity of the cell, such as its enormous size, or a low permeability.

The Nutrition of the Non-Nucleated Cell.

Since, as was shown above, glucose prolongs the life of amebas, and apparently acts as a food, it would be interesting to know whether it has a similar effect upon the cell from which the nucleus has been removed. To determine this, the nucleus was removed from a number of amebas, care being taken to remove as little protoplasm as possible. Under favorable circumstances it is possible to cut the nucleus from the cell without removing more than one-tenth of the protoplasm. Some of the amebas were now transferred to water, while others of equal size were kept in glucose solutions. The usual precautions were taken to prevent the development of bacteria.

Date	Number of amebas living	
	Water	0.5% glucose
Dec. 3	9	9
4	9	9
5	9	9
6	9	9
7	9	9
8	9	9
9	9	9
10	9	9
11	8	8
12	4	7
13	3	6
14	2	4
15	0	3
16		1
17		0
Average life-	8.9 days	10.2 days

Altho the amebas kept in glucose lived somewhat longer than the controls, the effect was not very striking. Since there are great differences between different amebas, even when selected from the same culture, a somewhat more satisfactory experiment was performed as follows: The nucleus was removed from a number of amebas, and then the amebas were divided in half. One half was kept in water, as a control, while the other half was kept in a solution of glucose.

	Number of amebas living	
Date	Water	1% glucose
April 3	12	12
4	12	12
5	12	12
6	12	12
7	11	11
8	10	11
9	9	11
10	6	10
11	5	6
12	2	4
13	0	3
14		0
Average life-	6.0 days	7.7 days

The non-nucleated cells live longer in glucose solutions, and probably use it as a food. This is in agreement with the work of Rous and Turner (1915), who used glucose for the preservation of the non-nucleated red

blood corpuscles.

If the life of the non-nucleated cell is prolonged by the use of glucose, is it further prolonged by the use of urea or ammonium nitrate ?

The nucleus was removed from 29 amebas, and after washing, ten were placed in glucose, ten in glucose + urea, and nine in glucose + ammonium nitrate.

	Number of amebas living		
Date	Glucose	Glucose + 0.1% urea	Glucose + 0.01% NH_4NO_3
Nov. 19	10	10	9
20	10	10	9
21	10	10	9
22	10	9	9
23	10	9	8
24	10	7	8
25	10	5	8
26	9	2	7
27	6	1	5
28	5	0	2
29	4		2
Dec. 2	0		1
3			0
Average life-	8.6 days	6.3 days	8.2 days

Thus in the non-nucleated cell, the addition of urea to the glucose was not beneficial: on the contrary it was harmful. The addition of ammonium nitrate was without effect.

Evidence was offered above that the normal, nucleated ameba was able to form some combination between glucose and urea (or some derivative of urea). If it is true that the beneficial effect of glucose + urea depends upon a synthesis, we may conclude that the non-nucleated cell is unable to perform this synthesis.

Respiration in the Non-Nucleated Cell.

It has been suggested (Loeb, 1905) that the nucleus is the organ of oxidation of the living cell; and that peculiarities of the non-nucleated cell, such as lack of the power to synthesize or to regenerate lost parts, are the result of a lowered oxidative activity. The non-nucleated pieces, it is said, "die slowly from asphyxia."

If this theory is true, we should expect to find a marked difference between the effect of depriving the non-nucleated cell of oxygen, and the effect of depriving the nucleated cell of oxygen. We should expect the cell in which oxidations were occurring most rapidly to be most affected by its removal, whereas the cell which was using very little oxygen should not be greatly affected. According to Child (1915), regions in which respiration is rapid are more susceptible to lack of oxygen, and die sooner than regions in which respiration is slower.

The effect of depriving the nucleated and non-nucleated cells of oxygen was investigated in the following manner: Five or more amebas were divided into two pieces of as nearly equal size as possible. After leaving them for a longer or shorter period in air, they were placed

in hanging drops, side by side, in an Englemann gas-chamber. Nitrogen, which had been washed in water, was then passed thru the chamber, and this atmosphere was maintained until the death of the organisms. In the third experiment, the nitrogen was first bubbled from a capillary tube thru strongly alkaline pyrogallic acid, to remove possible traces of oxygen. In the first experiment, the temperature of the water jacket surrounding the chamber was raised to 26° C. in order to accelerate the experiment. The other experiments were performed at 20° C.

	Time	Number of amebas living	
		Nucleated	Non-nucleated
Exp. 1	5:00 P.M.	Amebas	divided
	9:50 A.M.	Placed	in nitrogen
	10:00	7	7
	11:00	7	7
	12:00	7	6
	1:00 P.M.	7	4
	1:20	7	3
	1:30	6	1
	2:00	4	0
	2:25	3	
	2:30	2	
	2:35	1	
	2:45	0	
	Average life-	4.2 hrs.	3.1 hrs

	Time	Number of amebas living	
		Nucleated	Non-nucleated
Exp. 2	10:30 A.M.	Amebas	divided
	11:05	Placed	in nitrogen
	12:00	5	5
	1:00 P.M.	5	5
	2:00	5	5
	3:00	5	5
	4:00	5	5
	5:00	5	5
	6:00	5	5
	7:00	5	4
	7:30	5	2
	8:30	4	2
	9:00	4	2
	10:00	4	1
	11:00	4	1
	12:00	4	1
	8:00 A.M.	3	1
	9:00	3	1
	9:30	3	0
	10:00	3	
	10:15	1	
	11:00	1	
	12:00	1	
	1:00 P.M.	1	
	2:00	0	
	Average life-	17.5 hrs	11.7 hrs
Exp. 3	9:00 A.M.	Amebas	divided
	10:20	Placed	in nitrogen
	11:00	5	5
	12:00	5	5
	1:00 P.M.	5	5
	3:00	5	4
	4:00	5	2
	5:00	5	2
	8:00	5	1
	9:00	4	1
	9:30	4	0
	10:00	2	
	11:00	2	
	12:00	2	
	6:00 A.M.	0	
	Average life-	15.4 hrs	7.7 hrs.

Thus there is a distinct difference in the susceptibility to lack of oxygen in the nucleated and non-nucleated cell, but this difference is just opposite to what we should expect if oxidations were proceeding more rapidly in the nucleated half. Not only does the non-nucleated cell die more quickly when deprived of oxygen, but it is the first to assume a spherical shape and to cease putting out pseudopods.

The opposite experiment, that of increasing the supply of oxygen, is also of interest. Were it true that in removing the nucleus we have removed the organ of oxidation, and that the cell is slowly dying of asphyxia, it should be possible to delay the death of the non-nucleated cell by supplying it with more oxygen. On the contrary, it was found that when the nucleated and non-nucleated halves of amebas were kept in an atmosphere of oxygen, both died in less than twelve hours, and the non-nucleated pieces were killed quite as rapidly as those which contained nuclei.

The experiment was performed in the following manner: Five amebas were cut in half with a fine glass needle, and each half was transferred to a hanging drop in a gas chamber. Oxygen (Linde Air Products) which had been thoroly washed was now passed thru the chamber, and

the organisms kept under observation until all had disintegrated. The animals were divided at 10 A.M. Oxygen was passed thru at 10:30 A.M.

	Time of death	
	Nucleated cell	Non-nucleated cell
	1:30	2:00
	2:00	2:10
	4:15	3:23
	5:00	3:27
	6:30	4:15
Average life-	5.3 hours	4.5 hours

The injurious action of oxygen, which is doubtless the result of an increase in the oxidations of the cell, took effect somewhat more rapidly upon the cell which lacked a nucleus. It is difficult to see how a cell could be killed in a few hours by an atmosphere of oxygen if its oxidations have become depressed.

The Effect of Temperature upon the Non-Nucleated Cell.

In the experiments hitherto reported, a fairly constant temperature of 20° C. had been maintained. An attempt was now made to determine the effect of temperature upon the nucleated and non-nucleated cells, with the hope of discovering a possible difference in the rate at which chemical changes were occurring in the two cells. Two methods of investigation suggest themselves:

(1) The temperature of the medium is gradually raised until death occurs.

(2) The organisms are kept at a rather high temperature. and observed until death occurs.

Both of these methods were used in the order named.

In the first method, amebas were placed upon a Pfeiffer warming stage, thru which water of any desired temperature could be passed. After dividing the ameba into two equal parts, the temperature of the warming stage was raised at the rate of about one degree Centigrade per minute. The rate at which the temperature is raised is of importance, for by raising the temperature rapidly, the organisms may be killed at a comparatively low temperature. As the temperature rises, the amebas soon withdraw all of their pseudopods and assume a spherical shape. This does not, however, indicate the death of the organism, for if at this point the temperature is slowly lowered to 20° C., the animals will, in a few hours, send out pseudopods and resume normal movement. If the temperature is raised

high enough, the cells will either disintegrate completely or coagulate, with the result that the cell boundary can no longer be seen. The temperature at which the spherical shape was assumed, and the temperature at which death occurred are recorded below.

Exp.	Spherical shape assumed		Death	
	Nucleated	Non-nucleated	Nucleated	Non-nucleated
1	39° C.	38° C.	51° C.	50° C.
2	40	39	50	50
3	40	40	47°	52°
4	39°	44	49°	48°
Average-	39.5	40.2	49.2	50.1

The effect of gradual cooling was not so easily determined. When the temperature of the fluid was lowered greatly and even super-cooled, there was no injury provided crystallization did not take place. When the solution crystallised, however, as the result of adding a small crystal of ice, the amebas were injured mechanically, and disintegrated as soon as the solution melted.

But the effect of cooling could be shown in a somewhat different manner. The nucleated and non-nucleated parts of an ameba were slowly warmed to 44° C.,and then cooled in ten minutes to 22° C. Two minutes later, both fragments disintegrated at the same time: the result of

the rapid cooling.

Thus no difference could be found in the susceptibility of the nuclear and non-nuclear fragments to a rise or fall of temperature. The other method was now employed: keeping amebas at different temperatures until death occurred.

Eleven amebas were freed from food particles and debris, and divided into two equal parts. Both the nuclear and non-nuclear fragments were kept in a moist chamber at an average room temperature of 20° C.

Exp.1. 20° C. Date	Number of amebas living	
	Nucleated	Non-nucleated
Oct. 10	11	11
11	11	11
12	11	11
13	8	10
14	7	6
15, A.M.	7	3
15, P.M.	5	2
16	4	2
17	4	2
18	4	2
19, A.M.	3	1
19, P.M.	1	0
20	0	
Average life-	5.2 days	4.3 days

Thus, as was shown before, the life of the non-nuclear fragment at ordinary temperature isalmost as long as that of the nuclear fragment.

Ten amebas were now divided and each placed in a moist chamber which was kept in a thermostat at 30° C.

Exp. 2. 30° C.

Date	Number of amebas living	
	Nucleated	Non-nucleated
Oct. 22	10	10
23	10	7
23	9	7
24	9	4
25	9	2
26	6	1
~~25~~	5	0
27	~~4~~	
29	3	
30	3	
31	1	
Nov. 1	0	
Average life-	5.2 days	2.1 days

Apparently the life of the nuclear fragments was not shortened by exposure to high temperature, but this was probably due to the use of healthier amebas in Experiment 2. Further experiments showed that the life of nucleated amebas was shortened by raising the temperature, but the life of non-nuclear amebas was shortened somewhat more.

Twelve amebas were now divided and placed in a moist chamber at 10° C. The moist chamber was allowed to warm slowly to room temperature before removing the slide for examination.

Exp. 5. 10° C.	Date	Number of amebas living	
		Nucleated	Non-nucleated
	Oct. 17	12	12
	18	12	12
	19	12	12
	20	12	12
	21	12	12
	22	12	12
	23	11	11
	24	11	11
	25	11	11
	26	11	11
	27	11	11
	29, A.M.	11	9
	29, P.M.	11	8
	30, A.M.	11	5
	30, P.M.	11	4
	31, A.M.	11	3
	31, P.M.	10	2
	1, A.M.	5	0
	1, P.M.	3	
	2	0	
	Average life-	12.6 days	10.8 days

The life of both fragments was prolonged by cold in this case, but in two other cases it was distinctly shortened. This may have been due to the use of different cultures.

If susceptibility to high or low temperature may be used as an index to the rate of metabolism, there is no very great difference between the two fragments. There is some evidence that cells in which metabolic processes are rapid show a greater susceptibility to high temperature than do cells with a low metabolism (Child, 1915, p 66). If this is true, there is some slight indication of a more rapid metabolism in the non-nuclear fragment.

Metabolic Rate in the Non-Nucleated Cell.

The rate at which metabolic processes are occurring in different parts of an organism or tissue has been very successfully studied by determining their susceptibility to various poisons, especially to potassium cyanide (Child, 1913, 1915). Child has brot together a great deal of direct and indirect evidence to show that, in general, regions of high metabolism are more susceptible to poisons than are regions of low metabolism. Accordingly, the susceptibility of the nucleated and non-nucleated cell was investigated with a view to determining how the rate of metabolism was affected by the removal of the nucleus.

The amebas were cut in half, and one hour after the operation they were transferred to a fresh $\frac{M}{50}$ solution of KCN upon a hollow-ground glass slide. The drop of solution was immediately covered with a cover slip to prevent the escape of HCN. Since this could not be entirely prevented, the solution gradually became weaker, and as a result, those amebas which were not killed in two or three minutes usually lived for more than an hour. Since small fragments of ameba were found to be more susceptible than

large ones, an effort was made to divide the amebas into equal parts. After more than 50 amebas had been treated with cyanide, it was obvious that some unknown factor was playing a part in the death of the organisms: for altho the non-nucleated fragments usually succumbed first, there were many conspicuous cases in which the nucleated part died much sooner. It seemed possible that the unknown factor might be a difference between the anterior and posterior end. The amebas were divided when they were moving along the bottom in the "limax" condition, and in some cases the non-nuclear fragment was taken from the flowing anterior end, while in other cases it was taken from the retracting posterior end. In order to determine whether this made a difference, a number of amebas were divided in such a way that the nucleus remained in the anterior half, while others were so divided that the nucleus remained in the posterior half.

The number of minutes which the nuclear and non-nuclear fragments were able to withstand treatment with cyanide is tabulated below:

Duration of life

Nucleus in anterior end		Nucleus in posterior end	
Nucleated	Non-nucleated	Nucleated	Non-nucleated
96 min	8 min	190 min	83 min
5	34	155	10
45	15	145	5
200	200	85	1
190	190	170	167
45	170	5	5
260	240	145	115
1	2	146	2
290	530	105	2
580	62	100	1
6	180	98	7
2	140	5	3
260	1	1.5	1
260	25	1.9	0.8
300	15	76	2
230	3	74	2
230	140	6	2
320	305	80	11
87	2	80	10
230	6	75	5
185	41	1	0.5
255	450	12	1
450	250	9	3
2	445	25	25
4	100		
305	1		
10	1		
120	1		
1	0.5		
410	410		
330	330		
320	1		
Average 188.4 min	134.7 min	74.6 min	13.3 min

When the nucleus was in the anterior end, the non-nuclear fragment died first in 10 cases, the nuclear fragment died first in 7 cases, and in the remaining 4 cases each part disintegrated at about the same time. When the nucleus was in the posterior end, however, the nuclear fragment was never the first to disintegrate: the death of both fragments occurred at the same time in two instances, and in the remaining 22 cases the non-nuclear fragment was the first to disintegrate. The complicating factor is now understood. The anterior end of Ameba is more susceptible to cyanide than the posterior end. Essentially the same conclusion was reached by Miss Hyman (1917) in an investigation of the metabolic gradient in Ameba.

When the non-nucleated half is taken from the posterior end, its greater susceptibility to cyanide is not very definite because of the difference which exists between the anterior and posterior parts. When this disturbing factor is removed, however, by cutting the non-nuclear fragment from the anterior end, the non-nuclear fragment is seen to be much more susceptible to cyanide than the nuclear fragment.

The same difference in susceptibility between the nucleated and non-nucleated cells was found when they were kept for 20 hours after the operation before treatment with cyanide.

Whether we can conclude that the rate of metabolism is increased by removal of the nucleus is doubtful. It is possible that removal of the nucleus decreases the resistance to cyanides for some other reason. But at any rate, the evidence such as it is opposes the view that respiration and metabolism are depressed by removal of the nucleus.

Summary and Conclusions.

1. An ameba from which the nucleus has been removed may at times exhibit perfectly normal movement; in general, however, movement is somewhat affected by removal of the nucleus.
2. An ameba deprived of its nucleus lives almost as long as an ameba deprived of food.
3. Evidence is offered that Ameba can use glucose in solution as a food. There is also evidence that Ameba can synthesize glucose and urea, or some derivatives of these substances, to form a product which is of nutritive value.
4. Glucose is also of some benefit to the enucleated ameba, but the supposed synthesis of glucose and urea can not be performed.
5. The non-nucleated cell is injured more quickly by either a lack or an excess of oxygen than is the normal nucleated cell.
6. The non-nucleated cell is somewhat more susceptible to high or low temperature than the nucleated cell.
7. The non-nucleated cell is more susceptible to cyanide than the nucleated cell.

Discussion.

In the introduction, the two important theories of nuclear function were stated: the theory of synthesis and the theory of oxidation. According to the former theory, the non-nucleated cell is unable to construct, build up, synthesize new substances; while according to the latter, it is more or less unable to oxidise the substances already present. In reviewing the experiments here reported, it is seen that they not only offer strong evidence for the validity of the synthesis theory, but they constitute a practical proof that the oxidation theory is not true.

From the outset there has been no satisfactory evidence in support of the oxidation theory. It was apparently suggested by some experiments reported by Spitzer (1897). Spitzer obtained from various cells a preparation of nucleoprotein which contained a small amount of iron and which had the property of decomposing hydrogen peroxide. From this it has been concluded not only that all nuclei contain iron, but even that all nucleoproteins contain iron. The latter statement is of course false, and the microchemical evidence in support of the former statement (Macallum, 1892) is very unsatisfactory.* The presence of iron in the nucleus was

* From a very pure preparation of the sperm heads of the Great Lakes Whitefish, the author was unable to find a trace of iron in 0.5 gram of the dry material by the sulphocyanide test. The ashing was done in the electric muffle and by Neumann digestion, so that there was no question of "masked" iron. Unpublished.

taken as an indication that the nucleus played an important rôle in oxidations, but the presence of this iron is open to doubt. Spitzer's catalase may have been derived from the cytoplasm.

The chemical evidence for the oxidation theory is very slight, and the physiological evidence is equally so. The assumption that the organ which is furthest removed from the supply of oxygen is the organ of oxidation is not logical in the first place. "It should be born in mind that oxygen, in order to reach the nucleus, must penetrate a layer of cytoplasm containing reducing substances." (R. Lillie, 1911, p 722). Moreover, as was shown in the above experiments, the non-nucleated cell may exhibit perfectly normal movements. Since the energy for movement in aerobic organisms is derived from the oxidation of organic matter, it is unlikely that oxidations have become depressed. But if there is a depression of oxidations we should certainly expect (cf Loeb, 1905) to find some improvement in the cell when we increase its supply of oxygen. On the contrary, as has been shown, the cell becomes spherical and dies in a few hours. Also the susceptibility to lack of oxygen, the susceptibility to cyanide, and the susceptibility to high and low temperature all indicate that respiration and rate of metabolism are quite as rapid in the enucleated cell as in the normal cell.

Recently, however, experiments have been reported which

seem to give strong support to this theory: namely, the experiments of Osterhout on the leaf of the Indian Pipe. When the leaf of this plant is cut, the cells which have been injured soon become dark. Osterhout has shown that this darkening is an oxidation, and since it occurs first in the nucleus, argues that the nucleus is the center of oxidations. These experiments, however, admit of another interpretation. It is reasonable to beleive that at the center of the cell there is a strong reducing action, and that very little oxygen is present. After the death of the cell, however, oxygen, like other substances, may enter freely, and in the dying cell it would be expected that oxidations would be very rapid in the neighborhood of the nucleus, where the reducing action is greatest. In Osterhouts experiments, the cells which show the oxidative darkening of the nucleus are those which have been injured by cutting the leaf. Granted that the nucleus is the center of oxidations in the cell which is injured or dying, that does not prove that it is the center of oxidations in the normal living cell.

Moreover, Kite and Chambers (1912) found, in cells which were apparently uninjured, that reduction was most rapid in the region of the nucleus. The dye janus green becomes red (or colorless) when reduced. Kite and Chambers observed that this reduction occurred first at the centro-

some and second in the chromatin of the nucleus.

The experiments here reported offer strong support to the theory that the nucleus is the organ of synthesis. When a cell is deprived of food, the processes of synthesis must, to a great extent, come to a stop, altho synthesis of intermediary products of metabolism is still possible. It is probably not a coincidence that a cell deprived of its nucleus lives almost as long as a cell deprived of food.

An opportunity to put the synthesis theory to a direct test was afforded by nutritional experiments. When amebas are kept without food, they will die in from one to two weeks, but if glucose is present in solution their life is somewhat prolonged. It appears that the glucose is used as a food. When urea was added the organisms lived even longe , and in some cases divided. Urea alone, without glucose, had no such effect, a fact which is interpretted as meaning that the nitrogen compund is combined by the cell with the carbon compound. When nitrogen was already combined with unoxidised carbon, as in the amino-acids, the addition of glucose was unnecessary.

When these experiments were repeated upon the enucleated cell, it was found that glucose was somewhat beneficial, and was apparently used as a food. But the further addition of urea was not only of no benefit, but actually hastened the death of the protoplasm. Apparently non-nuclear protoplasm

is unable to combine carbon and nitrogen: the power of synthesis is absent.*

There is further evidence of the loss of the power of synthesis in the failure to produce the sticky secretion by means of which Ameba attaches itself to various objects. Normal amebas are firmly attached to the slide, and frequently stick to the sides of a pipette when drawn up into it. Non-nucleated amebas, however, shortly after the removal of the nucleus, are but lightly if at all attached to the slide, and never stick to the sides of a pipette.

In conclusion, the enucleated cell may move, respire, digest, respond to stimuli, and exhibit any activity which is dependent solely upon catabolic or destructive processes of protoplasm. The one group of phenomena which it never shows, are the phenomena of growth, and the related phenomena of regeneration and division. The phenomena of growth are essentially phenomena of organic synthesis, and the dependence of growth upon the nucleus involves the dependence of organic synthesis upon the nucleus.

* The configuration of atoms: C-N-C, so characteristic of the nucleic acid molecule, is also the configuration which occurs in the protein molecule at the points where the amino-acids are joined. This fact may be not without significance.

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